SUPPORT FOR THE AMENDMENTS

The description of the Drawings has been amended in accordance with the Examiner suggestions. The specification has also been amended with respect to trademarks as suggested by the Examiner. The amendments to the claims are supported by the specification, see page 2, last paragraph and the paragraph bridging pages 6 and 7. Claims 66-71 have been canceled. Accordingly, no new matter is believed to have been added to the present application by the amendments submitted above.

REMARKS

Claims 53-65 remain pending. Favorable reconsideration is respectfully requested.

The present invention relates to isolated and purified pili obtained from *Mycobacterium tuberculosis*, wherein the pili:

(a) are macromolecules which comprise pilin monomers, wherein the pilin monomers comprise proteins having a molecular weight of 14-25 kDa,

(b) are in the form of aggregated fibers, wherein the fibers have a width of 2 to 7 nm and a length of at least 5 microns,

(c) are immunogenic, and

(d) the aggregated fibers form a highly hydrophobic network.

The rejection of the claims under 35 U.S.C. §102(b) over Reed et al. (WO 97/09429 A2) is respectfully traversed. Reed et al. fail to disclose or suggest the isolated and purified pili.

Reed et al. disclose isolating polypeptides from *M. tuberculosis*. See the Abstract. The reference discloses SEQ ID NO: 5 of the present application. The Examiner has taken the position that SEQ ID NO: 5 is the same as the pili specified in Claim 53. This is incorrect.

The Examiner is correct that the sequence disclosed by Reed et al. is a 14 kd pilin monomer. However, this monomer is <u>not</u> aggregated to form pili as specified in Claim 53. Specifically, the protein described in Reed et al. is <u>not</u> (b) in the form of aggregated fibers, wherein the fibers have a width of 2 to 7 nm and a length of at least 5 microns and where (d) the aggregated fibers form a highly hydrophobic network, as specifically recited in Claim 53.

One major difference between the claimed pili and Reed et al. is that proteins described in the reference are peptide antigens isolated from Mtb culture supernatant which were soluble in water. This is in stark contrast to Mtp-- i.e., pili, which is a highly insoluble complex which the Inventors were unsuccessful in efforts to denature and solubulized in order to allow it to enter and run on SDS-PAGE gels for analysis. Mtp pili are supra-macromolecular structures that are formed by aggregates of dense fibrillar mesh-work, as described in the specification of the present application. Therefore the peptide produced by SEQ.ID NO. 5 or the small MW protein produced by this ORF is only a small pilin subunit of the Mtp pili. In comparison, Mtp is a massive mega-macromolecular structure, in which aggregated fibers form a highly hydrophobic network, as specified in Claim 53.

Contrary to the Examiner's assertions, the method of producing the claimed pili is different from the method described by Reed et al.

Reed et al. isolated soluble proteins (polypeptides) from the Mtb culture supernatant, either H37Ra or H37Rv, grown in GAS media at 37°C for two weeks. Please see pages 26-27. The recovered media was filtered to remove bacteria, and filtrate concentrated using an Amicon concentrator. Anion exchange column chromatography was then used followed by C18 HPLC. From this procedure they state they recovered 200 purified polypeptides, one being polypeptide SEQ. NO. 5, i.e. Mtp pilin. No evidence is presented to prove that these antigens were purified using as SDS-PAGE analysis and others.

Contrary to the Examiner's assertions to the contrary, there are many significant differences between the method to purify Mtp described in the present specification and the procedure described in Reed et al. First, Mtb H37Ra was grown under different culture conditions for optimal production of Mtp pili aggregates. H37Ra was grown on one hundred 7H11 agar plates supplemented with OADC at 37°C under 5% CO_2 for three weeks. The Mtb bacterial lawn was manually harvested into 150 mM mono-ethanolamine buffer. Then the

pili were released from the bacterial surface by mechanical shearing using glass beads. Using centrifugation the bacteria were separated from the pili-containing supernatant. The pili preparation was then extracted with 2:1 choloroform:methanol to remove vesicular material and lipids. The upper aqueous and interphase was observed to contain dense pili aggregates. This was recovered and further purified by ultracentrifugation. At each stage the presence of Mtp pili was verified by EM analysis.

Dr. Richard Friedman, one of the Inventors of the present application has repeatedly reviewed the Reed et al. application, and nowhere can he find any mention or evidence presented that they were the first to find pili in Mtb. According to Dr. Friedman, the most direct way to do that is to use transmission electron microscopy and negative stained bacteria to confirm the presence of Mtp pili, as described in the present application. Dr. Friedman also points out that the present application describes the use of EM at every stage of Mtp purification to confirm its presence. This procedure was not done nor described in the Reed et al. reference and their alleged statement that they used the protocol described in the present application, as described above, to purify Mtb or SEQ. NO. 5. is unfounded and unproven.

In the Office Action, the Examiner states "Since the prior art product [the Corixa application cited above] and the instantly claimed product have the same monomeric structure and the same *M. tuberculosis* origin and are both prepared by separation via mechanical shearing, the prior art product is expected to have the same characteristics or properties recited in claims 53, 55 and 66...A chemical composition and its properties and inseparable. Therefore, if the prior art teaches the identical chemical structure, the properties Applicants recite are necessarily present."

According to Dr. Friedman, the statement that both Mtp and their peptide were prepared and isolated by mechanical shearing to release the Mtp pili complex is untrue and erroneous. Neither is the method of isolation and purification set forth in the present application described in the Reed application nor use of EM to discover the presence in Mtp.

Dr. Friedman points out that on both page 5 and 6 of the Office communication, the Examiner states that Reed et al isolated and purified polypeptide antigens (i.e., pilin protein) with a MW of 14 kDa. At no place in the reference did Reed et al. discuss the isolation of Mtp and neither did they state that polypeptide SEQ.ID. NO: 5 were isolated using the procedure of mechanical shearing followed by centrifugation described in the present application.

Thus, Reed et al, did a 'fishing' experiment where they indiscriminately identified all of the soluble protein or protein fragments (peptides) that remained in the liquid medium following incubation with M. tuberculosis. This identification makes no assertion for function of any identified sequences.

More importantly, the methods used are clearly very different. Foremost, the Corixa patent describes the identification of soluble proteins from M. tuberculosis grown in liquid broth. The inventive method for purifying Mtp requires growth on a solid substrate (agar plates).

In addition, the protein fragment (peptide) that Reed et al identified, is only a small piece of the Mtp monomer. The Mtp monomer itself is only a small piece of the Mtp structure (by definition the monomer is the structural subunit).

In view of the foregoing, Reed et al. fails to disclose or suggest the claimed isolated and purified pili. Accordingly, the subject matter of the pending claims is not anticipated by or obvious over that reference. Withdrawal of this ground of rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §112, first paragraph, is believed to be obviated by the amendment submitted above. Claim 53 has been amended to specify that the fibers have a width of 2 to 7 nm and a length of at least 5 microns. Since this feature is described in the specification, withdraw of this ground of rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §112, second paragraph, is believed to obviated by the amendment submitted above. Claim 53 has been amended to specify that the fibers have a width of 2 to 7 nm and a length of at least 5 microns. In view of the foregoing amendment, the claims are definite within the meaning of 35 U.S.C. §112, second paragraph. Withdrawal of this ground of rejection is respectfully requested.

The objection to the specification is believed to be obviated by the amendment submitted above. The specification has been amended in accordance with the Examiner's suggestions.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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